6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring nickel, and other biomarkers of exposure and effect of nickel. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods may be included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

6.1 BIOLOGICAL SAMPLES

Analytical methods that determine nickel in biological materials are the same as those used for environmental samples. The most common methods determine the total nickel content of the sample instead of the particular nickel compound that may be present. Methodological differences are a function of the nickel level in the sample, digestion procedure required to solubilize the sample, and the level of potentially interfering substances that may be present. Either wet ashing with sulfuric acid or dry ashing through dissolution of the ash with dilute sulfuric acid is generally a satisfactory method to detect nickel in tissue or food (Boyer and Horowitz 1986). Digestion procedures for biological and environmental samples with particular reference to nickel determinations have been reviewed (Stoeppler 1980). As the digestion procedures require the use of strong acids, and substances with explosion hazards, for example perchloric acid, all safety procedures should be carefully reviewed before the analyses are completed.

Nickel is normally present at very low levels in biological samples. To determine trace nickel levels in these samples accurately, sensitive and selective methods are required. Atomic absorption spectrometry (AAS) and inductively coupled plasma-atomic emission spectroscopy (ICP-AES), with or without preconcentration or separation steps, are the most common methods. These methods have been adopted in standard procedures by EPA, NIOSH, IARC, and the International Union of

6. ANALYTICAL METHODS

Pure and Applied Chemistry (Brown et al. 1981). Direct aspiration into a flame and atomization in an electrically heated graphite furnace or carbon rod are the two variants of atomic absorption. The latter is sometimes referred to as electrothermal AAS. Typical detection limits for electrothermal AAS are <0.3 μg/L, while the limit for flame AAS and ICP-AES is 3.0 μg/L (Stoeppler 1984). The precision of analytical techniques for elemental determinations in blood, muscles, and various biological materials has been investigated (Iyengar 1989). Good precision was obtained with flame AAS after preconcentration and separation, electrothermal AAS, and ICP-AES.

Voltammetric techniques are becoming increasingly important for nickel determinations since such techniques have extraordinary sensitivity as well as good precision and accuracy. The addition of dimethylglyoxime, a chelating agent, to the electrolyte significantly enhances the method's sensitivity (IARC 1990; Stoeppler 1984). Detection limits less than 0.002 μg/L have been achieved with differential pulse anodic stripping voltammetry (DPASV) using dimethylglyoxime chelation.

Analytical methods and detection limits for nickel in biological materials are reported in Table 6-l. The presence of nickel in other biological materials such as hair and nails can be determined by the same analytical techniques used for blood and tissue after suitable procedures for dissolving the sample have been utilized (Stoeppler 1980; Takagi et al. 1986, 1988).

Detailed reviews regarding the methodology used to determine nickel in environmental and biological samples are available (Stoeppler 1980, 1984).

6.2 ENVIRONMENTAL SAMPLES

Analytical methods that detect nickel in environmental samples generally determine the total nickel content of the sample; determining specific nickel compounds is difficult. Filtering a water sample through a 0.45-μm membrane filter can distinguish between total and dissolved nickel (Martin et al. 1992). The most common methods used to detect nickel in environmental samples are AAS, either flame or graphite furnace, and ICP-AES. Nickel in water and waste water samples can be analyzed using EPA Test Methods 249.1 (atomic absorption, direct aspiration), 249.2 (atomic absorption, furnace technique), or 200.7 (ICP-AES) (EPA 1983), or the new direct current plasma atomic emission spectrophotometric method (EPA 1990b). Although these methods are suitable for groundwater and surface water samples and domestic and industrial effluents, the nickel

TABLE 6-1. Analytical Methods for Determining Nickel in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood fluid, tissue, and excreta[a]	Acid digestion in mixture of nitric, sulfuric, and perchloric acid	Electro-thermal AAS	0.2 µg Ni/L fluid; 0.49 µg Ni/kg of tissue	98% at 5 µg Ni/L; 97% at 8 µg Ni/L	IARC 1986 (Method 11)
Urine	Polydithiocarbamate resin extraction; ash filter and resins in a low temperature oxygen plasma asher or digest with HNO_3:$HCLO_4$	ICP-AES; NIOSH 8310	0.1 µg/sample	80%	NIOSH 1994b
Blood or tissue	Acid digestion in 3:1:1 (v/v/v) HNO_3:$HCLO_4$:H_2SO_4	ICP-AES; NIOSH 8005	10 µg/kg blood; 0.2 µg/g tissue	86% in blood	NIOSH 1994b

[a]If substantial quantities of iron are present (e.g., whole blood, tissues), hydrochloric acid is added, and the resulting ferric chloride is extracted with methyl isobutyl ketone.

AAS = atomic absorption spectrometry; $HCLO_4$ = perchloric acid; HNO_3 = nitric acid; H_2SO_4 = sulfuric acid; ICP-AES = inductively coupled plasma-atomic emission spectroscopy; Ni = nickel; NIOSH = National Institute for Occupational Safety and Health; v = volume

concentration in some groundwater, surface water, and drinking water is below the method detection limits. Therefore, the sample must be preconcentrated or other test methods must be used. Measurement of trace metals, including nickel in seawater can be completed using an in-line system with stripping voltammetry or chronopotentiometry (van den Berg and Achterberg 1994). These methods provide rapid analysis (1-15 minutes) with little sample preparation. The detection limit of these methods for nickel was not stated. Recommended EPA methods for soil sediment, sludge, and solid waste are Methods 7520 (AAS) and 6010 (ICP-AES). Before the widespread use of AAS, calorimetric methods were employed, and a number of calorimetric reagents have been used (Stoeppler 1980).

With analytical methods such as x-ray fluorescence (XRP), proton-induced x-ray emission (PIXE), and instrumental neutron activation analysis (INAA), many metals can be simultaneously analyzed without destroying the sample matrix. Of these, XRP and PIXE have good sensitivity and are frequently used to analyze nickel in environmental samples containing low levels of nickel such as rain, snow, and air (Hansson et al. 1988; Landsberger et al. 1983; Schroeder et al. 1987; Wiersema et al. 1984). The Texas Air Control Board, which uses XRP in its network of air monitors, reported a mean minimum detectable value of 6 ng nickel/m^3 (Wiersema et al. 1984). A detection limit of 30 ng/L was obtained using PIXE with a nonselective preconcentration step (Hansson et al. 1988). In these techniques, the sample (e.g., air particulates collected on a filter) is irradiated with a source of x-ray photons or protons. The excited atoms emit their own characteristic energy spectrum, which is detected with an x-ray detector and multichannel analyzer. INAA and neutron activation analysis (NAA) with prior nickel separation and concentration have poor sensitivity and are rarely used (Schroeder et al. 1987; Stoeppler 1984).

Contamination and loss are the main concerns when determining trace metals. Nickel-containing knives and needles should be avoided when collecting specimens. A study that compared the effects of using different dissecting tools on trace metal analysis did not report significant differences in the nickel content of fish or mussel samples dissected with stainless steel, lexan, titanium, or teflon-coated instruments (Iyengar 1986). Contamination can result from impurities in reagents or laboratory apparatus and laboratory dust. Losses may also occur when the analyte adsorbs onto container walls. When collecting air samples on filters, one should be aware that filter material can contain high and variable trace metal concentrations. Glass fiber filters may contain <80 ng/cm^2 of nickel. Silver membrane, cellulose, and polystyrene filters may contain

≈100 ng/cm^2 of nickel (Schroeder et al. 1987). Trace metals in blanks of different filter types and in different filters of the same type may vary from 5% to 20% (Brzezinska-Paudyn et al. 1986).

Some investigators have characterized the forms of nickel in an environmental sample by using successively stronger solvents. Each fraction solubilized is subsequently analyzed for nickel by atomic absorption or other procedures. In air, where the speciation of nickel is less complex, a method of sequential selective leaching has been developed to determine the amount of nickel in four phase categories of a dust sample, namely, soluble nickel, sulfidic nickel, metallic nickel, and refractory nickel oxides (Zatka 1990). Soluble nickel salts, mostly nickel sulfates, are leached at pH 4; sulfidic nickel is next solubilized with a peroxide-citrate solution; and metallic nickel is oxidized with bromine. The residue consists of refractory nickel oxides. Wong and Wu (1991) used an adsorptive stripping voltammetry method to determine different forms of nickel in air at a nickel manufacturing facility. The method distinguished between metallic nickel ions and nickel oxides. The results showed that speciation of nickel from several samples taken at the same location were highly variable. Although it is important to characterize the nickel contained in an environmental sample, methods that determine nickel speciation are difficult and not in widespread use.

Analytical methods and detection limits for standard methods of determining nickel in environmental media are reported in Table 6-2. If the determination of dissolved nickel is required, samples should be filtered with a 0.45-µpm membrane filter.

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of nickel is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of nickel.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if

TABLE 6-2. Analytical Methods for Determining Nickel in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air, airborne particulates	Collection on cellulose acetate filter; digestion with concentrated nitrated and perchloric acids	ICP-AES, NIOSH 7300	1 µg/ sample	105% at 2.5 µg; 97% at 1 mg	NIOSH 1994b
Water	Acid digestion in mixture of nitric, sulfuric, and perchloric acids	Electro-thermal AAS	0.2 µg Ni/L fluids	98% at 5 µg Ni/L; 97% at 8 µg Ni/L	IARC 1986 (Method 11)
Water, waste water	Acid digestion	AAS, direct aspiration; Method 249.1	0.04 mg/L	100% at 0.20 mg Ni/L; 97% at 1.0 mg Ni/L; 93% at 5.0 mg Ni/L	EPA 1983
	Acid digestion; sample solutions should contain 0.5% HNO_3	AAS, direct aspiration; Method 249.2	1 µg/L	Not applicable	EPA 1983

TABLE 6-2 (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
	Filter and acidify sample	ICP-AES; Method 200.7	15 µg/L	Accuracy: 6.7% at 30 µg/L; 8.3% at 60 g/L; 2.0% at 120 g/L	EPA 1983; Martin et al. 1992
Soil, sediment, sludge, solid waste	Digestion with nitric and hydrochloric acids; Method 3050	ICP-AES; Method 6010	15 µg/L	93±6%	EPA 1986b
Soil, sediment, sludge, solid waste	Digestion with nitric and hydrochloric acids; Method 3050	AAS, direct aspiration; Method 7520	0.04 mg/L	100% at 0.2 mg/L; 97% at 1.0 mg/L; 93% at 5.0 mg/L	EPA 1986b
Soil (total nickel)	Digest with nitric acid; oxidize with hydrogen peroxide at 450°C to destroy organic matter; digest with sulfuric and hydrofluoric acids, followed by digestion with nitric, sulfuric, and perchloric acids	AAS	0.02 µg/mL	No data	Baker and Amacher 1982

TABLE 6-2 (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil (DPTA extractable)	Shake soil with 0.005 *M* DPTA extraction solution for 2 hours	AAS	No data	No data	Baker and Amacher 1982
Soil (acid extractable)	Shake soil with 0.1 *N* hydrochloric acid for 5 minutes; complete 3 times	AAS	No data	No data	Baker and Amacher 1982
Food	Wet oxidation with sulfuric acid, complexation with ammonium tetramethylenedi-thiocarbamate followed by extraction with methyl butyl ketone[a]	AAS	20 µg/kg	No data	IARC 1986 (Method 17)

[a]The digestion procedure is not satisfactory for fats and oils. For these substances, sulfuric acid and 50% hydrogen peroxide should be used.

AAS = atomic absorption spectrometry; DPTA = diethylenetriamine pentaacetic acid; HNO_3 = nitric acid; ICP-AES = inductively coupled plasma-atomic emission spectroscopy; Ni = nickel; NIOSH = National Institute for Occupational Safety and Health

met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Nickel concentrations in hair, nails, blood, or urine are elevated in exposed individuals. A correlation has been established between nickel levels in urine, plasma, and feces in occupationally exposed workers and nickel levels in air (Angerer and Lehnert 1990; Bemacki et al. 1978; Hassler et al. 1983). If the identity of the nickel compounds to which workers are exposed is known, nickel levels in urine and plasma can be used as a biomarker for nickel exposure (Sunderman 1993). Available analytical methods can determine the nickel levels in these media in both unexposed and occupationally exposed persons. Methods to determine nickel speciation in biological media require further development.

There are no unique biomarkers of effect for nickel.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining total nickel in environmental media are well developed and adequate. Standardized methods are available from several sources including EPA (EPA 1983, 1986b). Most analytical methods measure total nickel content. Sequential extraction techniques are sometimes used to determine the nature of nickel in particles, e.g., they are exchangeable, adsorbed, easily reducible, or organically bound (Rudd et al. 1988; Rybicka 1989). There is a need for more development in this area and the adoption of standard methods for determining nickel species or forms of nickel in various media.

6.3.2 On-going Studies

The Nickel Producers Environmental Research Association (NiPERA) is sponsoring research on the application of inductively coupled plasma-mass spectroscopy (ICP-MS) to isotopic analysis of nickel in biological samples, on the development of sampling instrumentation for assessing workers' exposure to nickel in the nickel industry, and on methods for utilizing newly developed analytical

methods, such as iaser beam ionization mass spectrometry, for the identification and speciation of nickel compounds in powders and dusts with particular reference to nickel refining.